# AN EXPERIMENTAL STUDY ON THE REGENERATION OF PERIPHERAL NERVES.[1]

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The array of evidence is constantly accumulating on the one side, of the multi-cellular origin of the regenerative process, and on the other side of the growth of the newly formed nerve fiber from a central nerve cell. I have made a number of experiments, principally on rabbits, twenty-one of which were successful.

In this paper I have omitted the details of the histological examination in respect to the process of degeneration and regeneration, as these have been well described by many authors and usually in a similar manner. I have given simply an outline of the experimental work and the results obtained.

## EXPERIMENTS.

*Cases where union had taken place:*

12 hours.

Nerve cut entirely through. Axis cylinders do not stain as well in distal portion as in proximal. Some of the methods used have been:—

Weigert Pal stain.

Stroebe stain for axis cylinders.

8 days.

Cut anterior 2-3 of nerve, fibrous union. Stained with Mallory differential connective tissue stain. Some axis cylinders small and contracted, others swollen and vacuolated, all showing signs of degeneration.

5 weeks and 2 days.

Cut entirely through, partial union. There is some debris, degenerated myelin, etc., not yet absorbed and marked infiltration of neurilemma cells. No distinct axis cylinders in the specimens in the distal part.

9 weeks.

Cut out ½ of nerve, leaving a strand to guide union. On killing animal found bulbous union with axis cylinders and myelin sheaths growing through and appearing in distal portion.

10 weeks and 1 day.

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[1] This work was done at the Sheppard and Enoch Pratt Hospital, Baltimore, Md. Read before the Philadelphia Neurological Society, March 28, 1904.

Cut made entirely through. Very slight union, but axis cylinders were found in extreme distal part. Ink stain brought out fibers distinctly. Arnold's writing ink makes a fairly good selective stain for axis cylinders when tissue is hardened in Müller's fluid. The specimens are cut, placed in ink six days and differentiated as in Weigert Pal.

10 weeks and 6 days.

Cut nerve entirely through. On examination, space between ends was filled with a white scar tissue. This had formed a union and many fibers were seen in the tissue. They decreased in number the further they were removed from the point of union in the distal part.

Black pup. 11 weeks.

Large portion taken out but the space had been bridged over and fibers were easily demonstrated in peripheral portions. Lemon juice and gold make a most excellent stain for embryonic axis cylinders.

15 weeks 6 days.

Nerve cut entirely through. At autopsy the two ends were connected by a thin pale strip of tissue. This proved to be sufficient to convey fibers, as they were easily demonstrated in the distal part.

16 weeks.

Cut entirely through. Good union. Axis cylinders found throughout, myelin not well brought out.

17 weeks.

Slight union. Fibers found in distal part.

18 weeks.

Nerves cut entirely through. At autopsy there was a bulbous union and fibers were found in extreme distal part.

23 weeks.

A good union had taken place and well developed fibers were found in distal segment.

8 months.

Cut entirely through, which resulted in good union with recovery of function. Axis cylinders and myelin sheaths found well developed in distal portion.

*Cases where no union had taken place:*

6 weeks.

Cut out ⅜ inch. No union and no new fibers in distal portion. Most authors show fibers at fourth week. There are slight remains of degenerated myelin, which is the only change seen from distal portion of experiments many weeks older. The central end always gives the appearance of an endeavor to grow in all directions, many neurilemma cells, and new fibers intermixing and often forming a bulb. Ballance and Stewart claim to show new formed fibers at third week and that they stain readily in Weigert. Weigert and other stains in my cases showed absolutely no fibers where no union had taken place.

6 weeks 3 days.

Cut entirely through; at autopsy, no connection between ends whatsoever, and no fibers in distal segment, though easily demonstrated in proximal segment which ended in a bulbous formation.

7 weeks 3 days.

Cut out ⅜ inch at operation, no union. In distal part there are no fibers whatsoever. In all experiments there are in neurilemma cells lines suggestive of developing axis cylinders, but at no stage have I seen any development of them; they are granular, but never the full length of the cell. There is a strand of nervous tissue, newly formed, running from central end to the muscle continguous to it.

8 weeks.

¾ inch taken out at operation. No union and no vestige of new fibers in distal parts. This was twice the length of time required by Ballance and Stewart to show fibers.

9 weeks.

Cut out ⅜ inch of nerve, no union. No signs of any fibers in distal part, neurilemma cells show no change. A fiber of nervous tissue had grown from central end into near-by muscle.

10 weeks.

Cut entirely through. The union was by a slight band of connective tissue. No nerve fibers in union and none seen anywhere in distal part.

10 weeks.

Cut entirely through, no union. No nerve fibers in distal parts.

13 weeks.

Nerve cut, no union. No fibers found in distal parts.

In these experiments in every case where any union had taken place between the central and distal ends, newly developed fibers could be demonstrated in the peripheral part, but where there was no union between the two parts there was no axis cylinder or myelin sheath in the distal part.

Effort was always made to bridge over the gap and this occurred in thirteen of the experiments.. In the remaining eight where no union took place and there were no fibers in distal part, there was effort to bridge over; and in two cases (one at seven weeks) there was a distinct strand of nervous tissue running from the central end into the surrounding muscle, and in all cases there was formed a bulbous appearance in which were many new fibers intertwining with connective tissue and neurilemma in an effort to burst forth in every direction. No such appearance as this was seen in relation to the distal part. After five weeks there seems to be no change whatsoever in a distal unconnected part, it seems to have been prepared by proliferated neurilemma to form a path for the oncoming nervous process, but showed at no stage any signs of preparing the fiber itself.

Ballance and Stewart, in their extensive work on the subject, claim that the new fibers are formed from these proliferated neurilemma cells, but they are not careful to show whether the two ends may not have joined together.

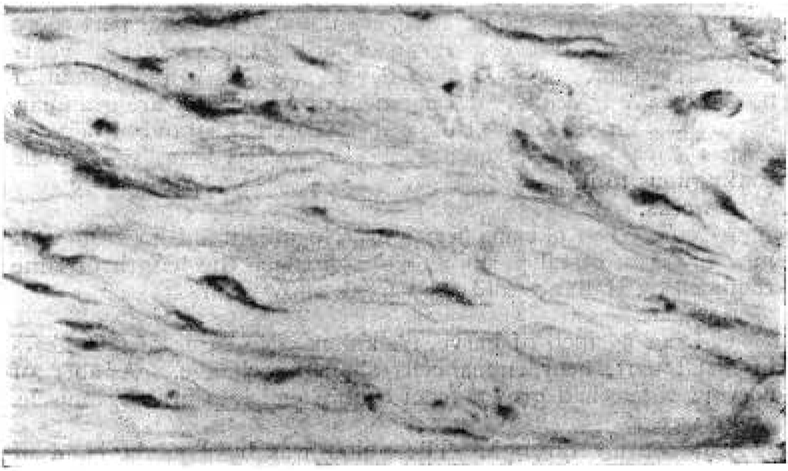
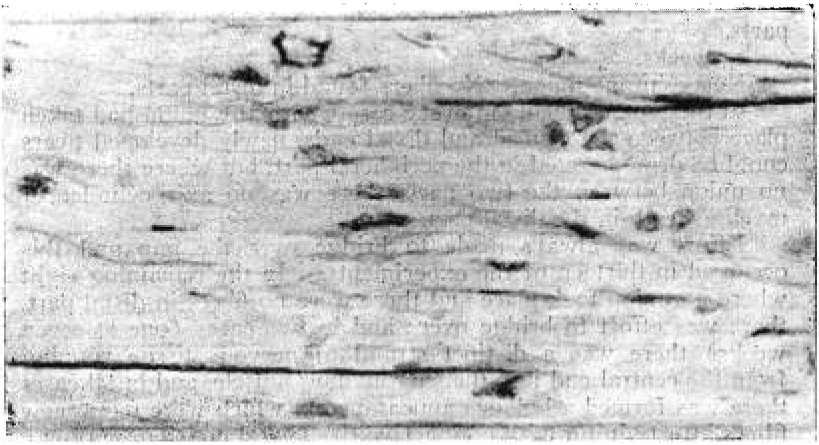


FIG. 1.—Distal portion. No union of two ends of nerve. No new fibers. 13 weeks.



10 weeks.
FIG. 2.—Distal end below union of two ends. Showing growing fibers.

Howell and Huber reached the conclusion that there was no peripheral regeneration. Langley and Anderson,[2] also in this country during the past year working with cats and rabbits, insist that nerve fibers isolated from the spinal cord and spinal ganglia do not reform medulated fibers. In another series of experiments they show that the central end of any efferent fiber can make functional connection with the peripheral end of any other efferent fiber of the same class, whatever may be the normal action produced by the two nerve fibers. They also show that where the peripheral ends of two divided nerves are joined, there is no union. It would seem that if there were peripheral regeneration that these latter would join in some way.

Spiller and Frazier[3] have shown that there is probably no regeneration centralward of posterior spinal roots. This seems to fall in line with the possible idea that neurilemma cells have to be present to prepare a path; but at the same time it does not argue against neurilemma cells being the origin of a multicellular regeneration, for the examples are few of regeneration of the spinal cord where there are no neurilemma cells.

In England, during the last year Mott, Halliburton and Edmunds[4] deny all possibility of the formation of axis cylinders by neurilemma cells. The clinical observations of early return of sensation after suture are not considered trustworthy by them.

At the Oxford meeting of the British Medical Association, last September, Robert Kennedy[5] read a paper in defense of the peripheral regeneration theory. He had examined peripheral segments of nerves up to eighteen months after their division and found the histological characters to have been the same as in young nerve fibers. He implanted a portion of a nerve subcutaneously and in it even found newly formed fibers.

This fact is usually explained as being due to penetration of fibers from the cut end of the nerve seeking the isolated portion. These experiments may be true, but there are many ways of committing error. Kennedy used Stroebe's stain, which is very difficult of certainty. In the Weigert Pal method there is often an arrangement of neurilemma nuclei in a line which would give the appearance of a new fiber, while really it is not, and such arrangements are not uncommon.

Kennedy says if there were such an affinity for nerve fibers to seek out nerve tissue as in a subcutaneous grafting there would be no reason for aiding nature in repairing a divided nerve. We know that it is necessary to suture in order to get good union, and I feel from my own experiments that without union, no matter how distant from a central end of an efferent nerve, there will be no fibers in the peripheral part. Of course there is the careful work of Bethe[6] to be considered, which I do not feel competent to criticize. He worked principally with young animals, however, and did not prove conclusively that it was so in adults. I experimented on a number of a day-old kittens, but they grew so rapidly that I could not be convinced that the experiments were reliable. Another puzzling phenomenon is the return of sensation in two to three days in sutured nerves, which Kennedy claims to be a fact based on forty cases that he has examined. Mott and Halliburton and Edmunds were extremely skeptical regarding the accuracy of such observations.

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[2] Journal of Physics, Vol. 31, 1904.

[3] University of Penna., Med. Bul., June, 1903.

[4] Proceedings of Physiological Society, March 19, 1904.

[5] Brit. Med. Journal, Sept. 24, 1904.

Munzer[7] working in Prague repeated some of Bethe's experiments and found in a divided nerve only the sheaths of Schwann with proliferated neurilemma cells. He demonstrated in an ununited experiment that fibers would follow along the contiguous layer of muscle and enter the distal portion of a nerve. Such a condition as this I found in two cases, where well developed strands of new nervous tissue came from the central end and entered the adjoining muscle but did not reach the nerve.

At the meeting at which Munzer read his paper, M. Raimann said that he had repeated Bethe's experiments and had come to the same conclusion as Munzer.

To my mind it seems improbable that if neurilemma cells originate the new fibrils in the distal and central portions that we have connecting islets of new fibers in the scar tissue where we would not expect to find neurilemma cells.

No one has ever demonstrated a meeting or joining of these islets of so-called new fibers, but they assume that they do. If fibers are seen they would appear to me to be merely parts of fibers from central end cut obliquely in the sections or arrangements of neurilemma nuclei resembling new fibers, for with a Weigert stain they both take the same color.

A most convincing paper is that by Dr. R. G. Harrison[8] in which he demonstrates that in embryos of Rana esculenta the naked fiber is first laid down to the periphey and that the cells of Schwann migrate later from the ganglionic ridge and envelop them. He has observed the movement of Schwann cells from the center to the periphery along the course of a naked nerve fiber in the fin of an embryo. He maintains that the growth of the nerve is by ameboid movement of the protoplasm at the end of the nerve cell prominence as he has demonstrated in various embryos. This work would seem to argue in favor of central regeneration in healing nerves.

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[6] "Allgemeine Anatomie und Physiologie des Nervensystems."

[7] Neurologisches Centralbl., Oct. 16, 1902.

[8] Neiderheim Gesellsch. f. Natur. und Heilkunde zu Bonn, 1904.